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THE CAUSE OF AMERICAN FOUL BROOD.[1]

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For several reasons much confusion exists as to the present status of our knowledge of bee diseases. It is hoped that this circular will give information which will to some extent clear up the subject of American foul brood from a bacteriological point of view. The symptoms of this disease are given in Circular No. 79 of the Bureau of Entomology, entitled "The Brood Diseases of Bees." American foul brood is the prevalent disease in America, and, judging from reports received from Europe and from descriptions in European bee journals and books, it is the prevalent one there. There is another diseased condition, which Cheyne examined, and to which we now refer as European foul brood.

When the author began his work on bee diseases in the summer of 1902 he observed, in combs containing American foul brood, in the dried remains of the dead larvæ, known as the scales, a very large number of spores which failed to grow when inoculated into the media ordinarily used in the laboratory. It was clear, then, that these spores are not *Bacillus alvei* and that this disease is not the "foul brood" of Cheshire and Cheyne. The following year the study was continued and a medium was devised in which the spores found in this disease will germinate. This medium consists of an agar made by following the directions ordinarily used in the laboratory, with the exception that bee larvæ are substituted for meat. By the use of this medium were obtained pure cultures of the microorganism which is found so abundantly, in the form of spores, in the dried scales of American foul brood.

In reporting these findings the author referred to this organism as "*Bacillus X.*" Further study was subsequently made, and the species was given the permanent name *Bacillus larvæ*. The description of this species may be found in Technical Series No. 14 of this Bureau. In his publications the author has made no claim that *Bacillus larvæ* is the cause of American foul brood, but has made the statement that it is found to be present in all the samples of this disease which have

[1] The author wishes to acknowledge the assistance of Dr. E. F. Phillips, in Charge of Apiculture, under whose direction this work has been done. These results have been reached since the author has been connected with the apicultural investigations of the Bureau of Entomology.

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been examined by him. No inoculation experiments were made, for the reason that sufficient cultures in suitable condition could not be obtained from any medium then known.

Since the media used in former investigations are not suitable for obtaining cultures for purposes of inoculation, in taking up the further study it has been necessary to devise a medium which would be satisfactory in this respect. Such a medium has been discovered, and large amounts of the culture suitable for experimental inoculations have been obtained. This medium is prepared and used as follows: Healthy bee larvæ or young pupæ are picked from the comb, crushed, strained through cheese cloth, diluted with 20 to 50 times their volume of water, filtered through ordinary filter paper, and then passed through an earthenware filter (the Berkefeld filter is satisfactory) to remove any bacteria which are present. The sterile filtrate thus obtained may be pipetted into tubes or flasks and stored until needed. When *Bacillus larvæ* is to be isolated, a tube of the ordinary agar of the laboratory is liquefied and cooled to 45° or 50° C. Then about 2 c.c. of the filtrate mentioned above is added to it. A very small amount of the decaying larvæ affected with American foul brood is then added. The procedure from this point is as usual in making agar plate cultures; these plates are afterward incubated. When a large amount of culture is desired for experimental purposes it is convenient to use the ordinary agar medium in large test tubes to which has been added, as above, about 2 c.c. of the sterile larvæ filtrate. These agar tubes are then inclined and the surface of the congealed agar is inoculated. In no case should the larvæ or filtrate reach a high temperature. The object, of course, is to obtain a medium which contains the food constituents which are afforded the bacteria in the living larvæ.

Inoculation experiments have been made by feeding to a healthy colony the scales from combs which had contained brood affected with American foul brood. The result of the feeding was that the colony became affected by disease, the symptoms of which were the same as those observed in the apiary where American foul brood is found. Like symptoms have been produced by feeding scales which had been put into ordinary meat bouillon, incubated for twenty-four hours, and then heated to 65° C. for twenty minutes.

On microscopic examination of the decaying larvæ dead from the disease thus produced experimentally, the same large number of spores and rods are seen as when samples are examined which are taken from an apiary affected with American foul brood. From these dead larvæ pure cultures of *Bacillus larvæ* were obtained from plates, using the new medium described above. These experiments show that by the feeding method the disease may be produced and that the contagion is found in the scales. The second experiment tends to indicate that the

cause of American foul brood as found in the scales is not killed by heat at 65° C. applied for twenty minutes.

Up to the present time there is no authentic record of this disease having been produced by experimental inoculations of pure cultures.

Knowing that by the feeding method the disease may be produced, pure cultures of *Bacillus larvæ* have been mixed with sterile sugar sirup and fed to healthy colonies with the result that the disease appeared in the colonies within three weeks with symptoms identical with those produced by feeding the scales of the disease. In the ropy brown mass of the decaying larvæ in the disease which is produced experimentally by feeding pure cultures of *Bacillus larvæ* there are found the same large number of spores and rods as when the disease is produced by feeding the scales or when the disease is found in an apiary. Pure cultures of *Bacillus larvæ* have been obtained from the larvæ dead from the disease produced experimentally by feeding pure cultures of *Bacillus larvæ*.

Some European investigators of brood diseases omit the symptoms, so that it is impossible to tell which disease they are investigating. Their descriptions of microorganisms also are entirely too brief. These facts have led to much confusion, and they necessitate much additional work on the part of other investigators. They have also added to the present confusion. From what can be gained from their papers, the author is inclined to believe that Burri has been working with *Bacillus larvæ* and has been referring to it as the "bacillus difficult of cultivation;" that Maassen has been working with *Bacillus larvæ* and has been referring to it as *Bacillus brandenburgiensis*, and that von Buttel Reepen has referred to *Bacillus larvæ* as "*B. burri*." It is hoped that this confusion may soon cease to exist.

In the study of *Bacillus larvæ* on this new medium some interesting additional facts have been observed in the morphology and cultural characters of this organism which will be given in a bulletin from this Bureau in the near future. One fact is mentioned now because it seems to have caused one German investigator, Dr. Albert Maassen, to fall into error in the interpretation of certain findings. This fact is that this species, *Bacillus larvæ*, produces a large number of giant whips. (Giant whips are at present believed to be in some way a modification of flagella, the motile organs of bacteria.) These giant whips appear in pure cultures of *Bacillus larvæ* and persist there for a long time. The structures which Maassen evidently saw and reported in two different publications, naming them *Spirochæta apis*, are nothing other than giant whips which normally belong to *Bacillus larvæ* and which are formed by the growth of *Bacillus larvæ* in the larvæ of the bee.

Maassen seems to have no further evidence that the structures which he saw are spirochætes than what could be gained by a microscopic examination of the remains of the dead larvæ which had suffered from

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this disease. The appearance which he interprets as a spirochæte in the process of division can be seen in the giant whips obtained from pure cultures of *Bacillus larvæ*. These giant whips are found in the decaying larvæ which are dead from American foul brood experimentally produced by feeding pure cultures of *Bacillus larvæ*.

The author has observed these structures in a large number of examinations of American foul brood, especially in the hanging-drop preparations made directly from the dead larvæ. There is nothing else contained in the dead larvæ which can be seen that resembles a spirochæte, and since Maassen made no mention of the giant whips found there so abundantly, it is quite certain that he has made this mistake.

This preliminary note will be followed by a bulletin which will contain in full the results of recent investigations by others on the brood diseases of bees and a detailed account of the work done here.

The results may be summarized as follows:

(1) In previous publications the author has made no claim that *Bacillus larvæ* is the cause of American foul brood.

(2) A medium has been devised by which cultures of *Bacillus larvæ* may be obtained in large quantities suitable for experimental inoculation. This medium consists of the sterile filtrate obtained by diluting and filtering the crushed bodies of bee larvæ through a Berkefeld or other fine filter.

(3) American foul brood has been produced by feeding pure cultures of *Bacillus larvæ*, and the symptoms of the disease are the same as those produced by feeding the scales of this disease and as those observed in the apiary where colonies are affected with this disease.

(4) The structures described by Doctor Maassen, of Dahlem, Germany, as spirochætes and named by him *Spirochæta apis* are not spirochætes, but normal structures produced by the growth of *Bacillus larvæ*. These are known in bacteriology as giant whips.

Approved:

W. M. Hays,

Acting Secretary of Agriculture.

Washington, D. C., *July 15, 1907.*

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